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for Treatment of Mammary Carcinoma

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13. ABSTRACT (Maximum 200 Words) This project will evaluate paclitaxel chemotherapy delivery from a gel polymer system placed into a wound bed following conservative surgical removal of human breast cancers grown in nude mice. This novel delivery method is proposed to control local tumor disease as well as assist in control of metastasis and may offer a cost-effective alternative to adjuvant radiation therapy. Task (objective) 1 (proposed to be completed in year 1): To evaluate the efficacy of polymer delivered paclitaxel chemotherapy against human breast tumor cell lines. As per task 1, we have established 5 human breast cancer cell lines within our laboratory; MCF-7, MCF-7 AL, MDA-MB-435, MDA-MB-231 and MX-1. We have elected to pursue purchase and implementation of a unique luciferase imaging system (not in original proposal) which will allow in vivo imaging of tumor growth and metastasis (and subsequently decrease animal use). Use of this system requires transfection of the breast tumor cell lines with the luciferase gene. Thus far we have successfully transfected the MDA-MB-435 line and are in the process of transfecting the remaining cell lines. This has caused a slight delay in completion of task 1, however, we have recently completed in vitro testing of 4 of 5 of the cell lines for taxol sensitivity (results enclosed).				
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William S. Demell DVM, MS

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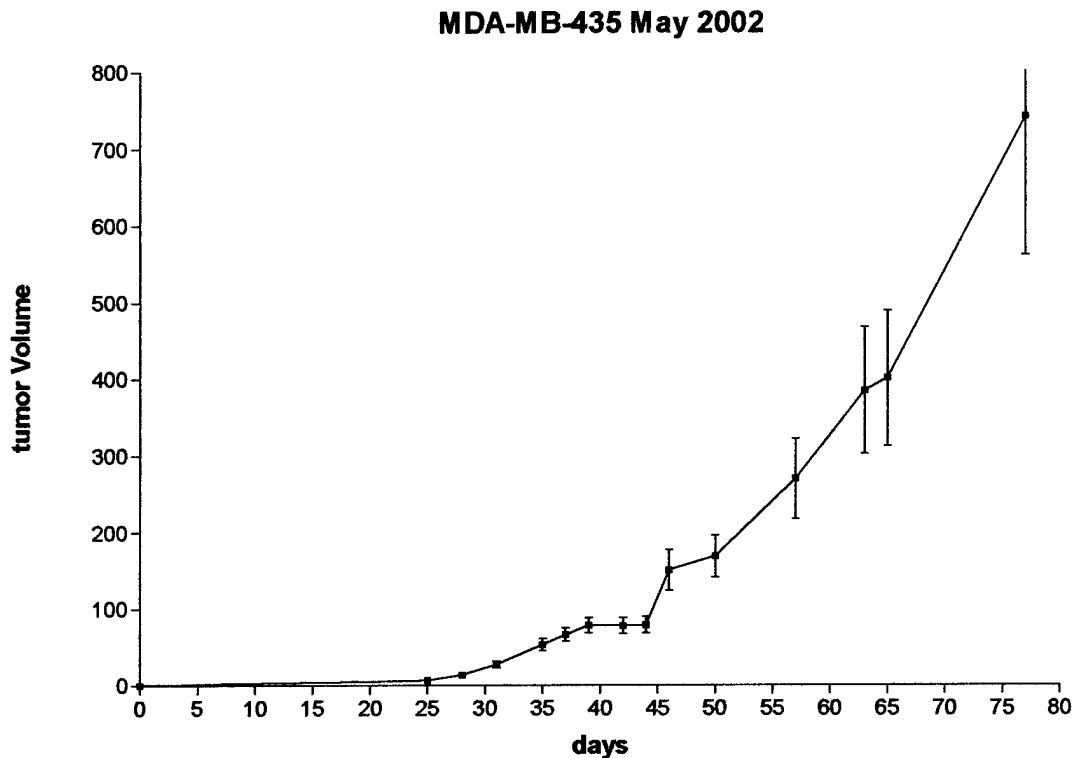
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William S. Dornell DVM, MS

Introduction: This proposal will evaluate paclitaxel (taxol) chemotherapy delivery from a gel polymer system placed into a wound bed following conservative surgical removal of human breast cancers grown in nude mice. This novel delivery method is proposed to control local tumor disease as well as assist in control of metastasis and may offer a cost-effective alternative to adjuvant radiation therapy.

Body: Task (objective) 1 (proposed to be completed in year 1): *To evaluate the efficacy of polymer delivered paclitaxel chemotherapy against human breast tumor cell lines.* As per task 1, we have established 5 human breast cancer cell lines within our laboratory; MCF-7, MCF-7 AL, MDA-MB-435, MDA-MB-231 and MX-1. We have extensively evaluated the MDA-MB-435 orthotopically xenografted model in vivo in mice and have obtained consistent growth (see Chart 1).

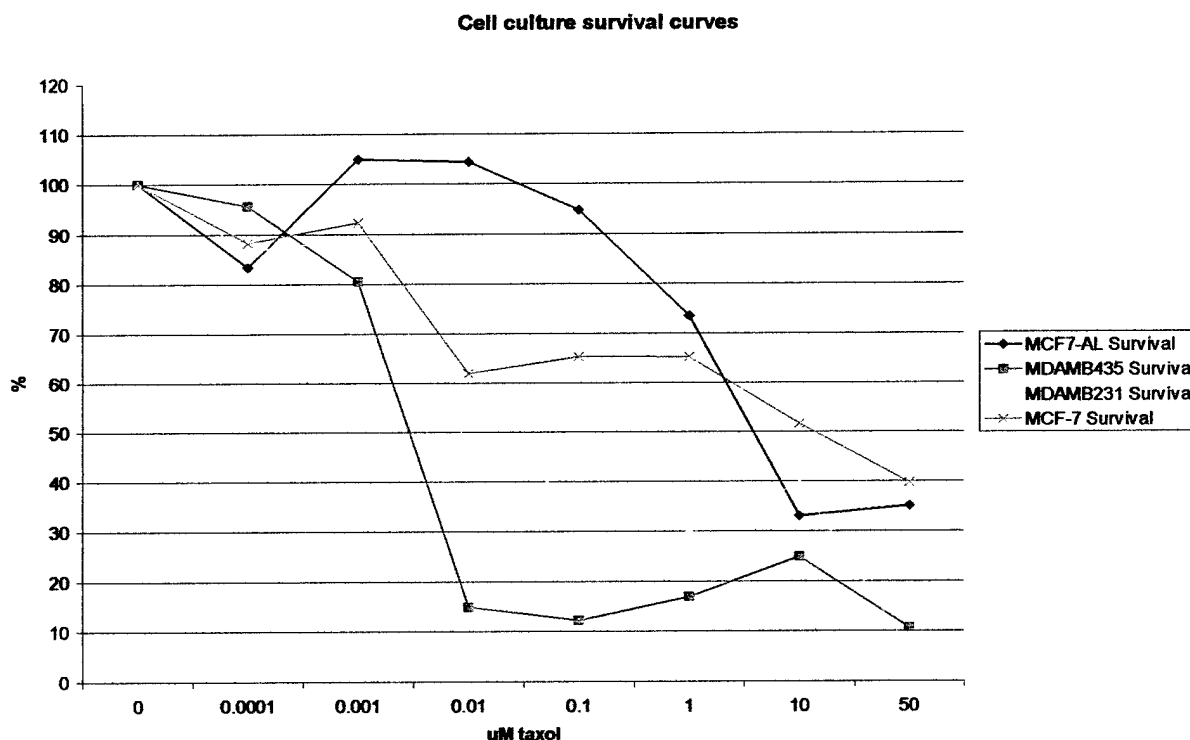
Chart 1. Tumor growth following injection of MDAMB435 cells in nude mice.



We have tested 4 of the 5 cell cell lines in vitro for sensitivity to taxol by MTS assay (*see Chart2*). An LC50 was found for each of the cell lines at the following concentrations:

- MDAMB435 (0.005 uM)
- MDAMB231 (0.01uM)
- MCF-7 (10 uM)
- MCF-7/AL (adriamycin resistant) (10uM)

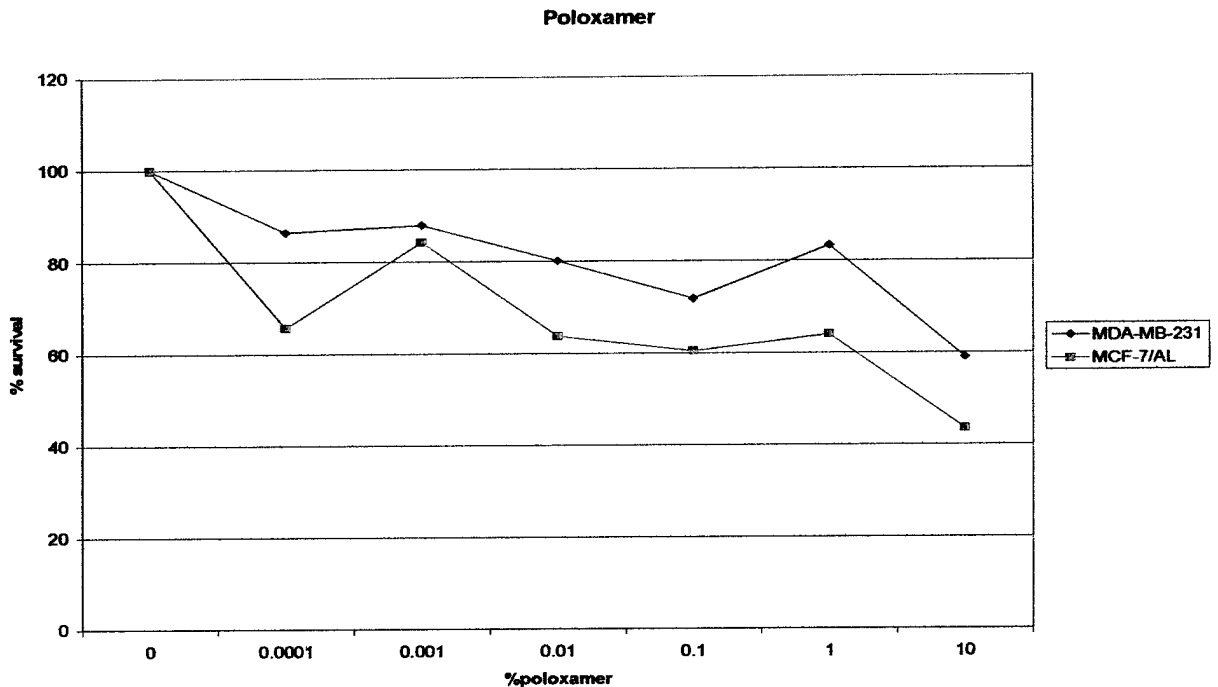
Chart 2. Cell culture survivability using the MTS assay.



In addition, we have tested both MDA-MB-435 and MDA-MB-231 by clonogenic assay in which the LC50 was found to be 0.01 and 0.1 μ M, respectively. The advantage of a clonogenic assay is that it allows you evaluate if the cell can still mitose, while with the MTS assay, you evaluate cell survivability only. Both these assays have demonstrated consistent sensitivity of these chosen cell lines to taxol.

To test the potential cytotoxic effect of the poloxamer gel polymer, which will be used to locally deliver the taxol (polotax), we used the MTS assay with MDA-MB-231 and MCF7/AL cells exposed to increasing concentrations of poloxamer (see **Chart 3**). This testing did show slight evidence of a cytotoxic effect of the carrier alone at higher concentrations. Our next step will be to test the poloxamer taxol combination (polotax) for cytotoxicity to the cell lines, comparing this to taxol alone and poloxamer alone. In addition, we plan to test intracellular concentrations of taxol within the cell lines tested, comparing taxol alone and the polotax. This will determine if poloxamer has any effect on P-glycoprotein mediated drug resistance.

Chart 3. Cell survivability following exposure to poloxamer 417.



We have elected to pursue purchase and implementation of a unique luciferase imaging system (not in original proposal and not paid for using grant monies from this award) which will allow in vivo imaging of tumor growth and metastasis. The following paragraph describes how this system will be implemented:

Tumor growth will be evaluated by IVIS technology. Briefly, animals will be anesthetized by i.p. injection of 40 ul of a ketamine and xylazine (4:1) solution. An aqueous solution of the substrate luciferin (the substrate for luciferase, Molecular Probes, 50mM, 126mg/kg) will be administered by intraperitoneal injection 5 min before imaging (Sweeney et al., 1999). Supine mice will then be placed into a light-tight specimen chamber mounted with the charge-coupled device (CCD)- camera cooled to -120°C. A gray-scale body-surface reference image will be collected first followed by acquisition of the photons transmitted from the luciferase transfected cells in the mice. Using LIVINGIMAGE software (Xenogen), overlay of the pseudocolor image will represent the spatial distribution of photon counts. Signal intensity will be quantified as the sum of all detected photon counts within the region of interest after subtraction of background luminescence measured at shoulder level (Vooijs et al., 2002).

Use of this system will allow evaluation of disease progression (and response to treatment without the need for animal sacrifice until the final endpoints of the study. This will significantly decrease animal use. Use of this system requires transfection of the breast tumor cell lines with the luciferase gene. We are currently transfecting the MDAMB435 cell line. We will then transfect the remaining (taxol sensitive) cell lines with the luciferase gene prior to moving on to task 2.

Task (objective) 2: *To evaluate the local and systemic toxicity of locally delivered (intracavitary; within the wound bed) paclitaxel chemotherapy following tumor removal. This work will be conducted in the upcoming year (year 2).*

Key Research Accomplishments:

1. Establishment of 5 (commercially available) human breast tumor cell lines within our laboratory.
 - a. MCF-7
 - b. MCF-7 AL
 - c. MDA-MB-435
 - d. MDA-MB-231
 - e. MX1
2. In vivo growth of MDA-MB-435 cell line in nude mice.
3. In vitro testing of cell lines for taxol and poloxamer cytotoxicity.
4. Beginning transfection of cell lines with the luciferase gene.

Reportable Outcomes: N/A

Conclusions: Establishment of the cell lines and testing for sensitivity to taxol chemotherapy and the poloxamer has brought us close to completion of task 1. We have one additional cell line to test and then will be testing the enhanced cytotoxicity of the poloxamer/taxol combination. The decision to obtain and utilize the in vivo luciferase imaging system has resulted in a slight delay in completion of task 1 due to the need for cell line transfection with the luciferase gene. We feel this is more than offset by the decreased use of animals and the implementation of this technology into this work.

References:

Sweeney TJ, Mailander V, Tucker AA, Olomu AB, Zhang W, Cao Y, Negrin RS, Contag CH. Visualizing the kinetics of tumor-cell clearance in living animals. *Proceedings of the National Academy of Sciences of the United States of America*. 96(21):12044-9, 1999.

Vooijs M, Jonkers J, Lyons S, Berns A. Noninvasive imaging of spontaneous retinoblastoma pathway-dependent tumors in mice. *Cancer Research*. 62(6):1862-7, 2002.

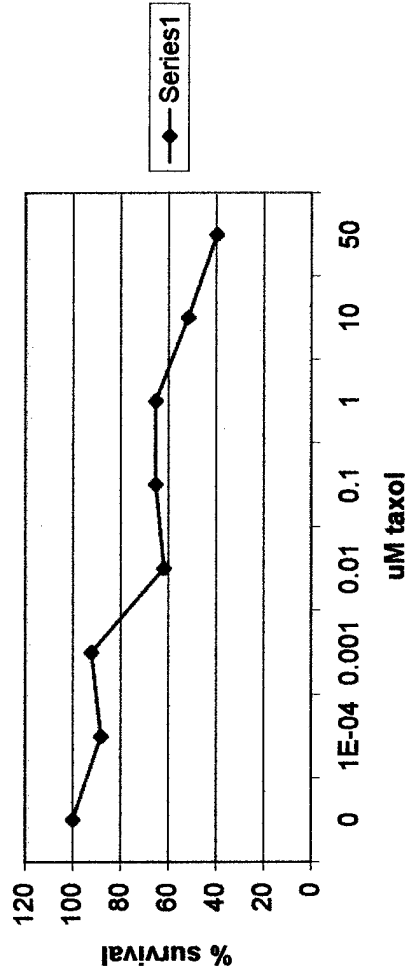
Appendix 1 (attached): Excel file of MTS assay results including sample cytotoxicity charts.

Appendix 1. DAMD17-02-1-0348 Human Mammary Tumor Cell Line MTS Cytotoxicity Data													
Survival ratio	0	0.0001	0.001	0.01	0.1	1	10	50					
	100	108.5331	128.9549	122.5312	92.52157	96.16491	39.88495	40.46021					
	100	82.9429	95.75403	99.12152	102.9283	67.20351	25.40264	30.08785					
	100	58.65315	90.3693	91.74511	88.77625	57.27734	34.03331	34.97466					
average	100	83.37638	105.0261	104.4659	94.74203	73.54859	33.10696	35.17424					
13-Jun Taxol													
231													
	no cells	EtOH	0	0.0001	0.001	0.01	0.1	1	10	50			
	-0.013	1.326	1.401	1.324	1.22	0.5	0.474	0.419	0.631	0.069			
	-0.022	1.389	1.229	1.033	1.033	0.626	0.443	0.369	0.377	0.07			
	-0.014	1.075	1.39	1.136	1.336	0.757	0.576	0.433	0.595	0.109			
Survival ratio	0	0.0001	0.001	0.01	0.1	1	10	50					
	100	94.50393	87.08066	35.68879	33.83298	29.90721	45.03926	4.925054					
	100	84.05207	84.05207	50.93572	36.04557	30.02441	30.67535	5.695688					
	100	81.72662	96.11511	54.46043	41.43885	31.15108	42.80576	7.841727					
average	100	86.76087	89.08261	47.02832	37.1058	30.3609	39.50679	6.154156					
13-Jun Taxol													
MDAMB435													
	no cells	EtOH	0	0.0001	0.001	0.01	0.1	1	10	50			
	1	2	3	4	5	6	7	8	9	10			
	-0.052	1.15	1.622	1.275	0.937	0.21	0.248	0.234	0.326	0.078			
	-0.034	1.658	1.842	1.921	1.672	0.299	0.201	0.463	0.489	0.367			
	-0.035	1.937	1.791	1.86	1.667	0.284	0.191	0.207	0.506	0.136			
Survival ratio	0	0.0001	0.001	0.01	0.1	1	10	50					
	100	78.60666	57.76819	12.94698	15.28977	14.42663	20.09864	4.808878					
	100	104.2888	90.7709	16.23236	10.91205	25.13572	26.54723	19.924					
	100	103.8526	93.07649	15.85706	10.66443	11.55779	28.25237	7.593523					
average	100	95.58269	80.53853	15.01213	12.28875	17.04005	24.96608	10.77547					
13-Jun Taxol													
MCF-7													
	no cells	EtOH	0	0.0001	0.001	0.01	0.1	1	10	50			
	0.073	1.223	1.234	1.181	1.085	0.668	0.802	0.592	0.698	0.395			
	0.02	0.936	1.094	0.98	0.998	0.621	0.616	0.648	0.577	0.375			
	0.028	0.709	0.786	0.625	0.769	0.59	0.587	0.696	0.359	0.417			

Appendix 1. DAMD17-02-1-0348 Human Mammary Tumor Cell Line MTS Cytotoxicity Data												
Survival ratio	0	0.0001	0.001	0.01	0.1	1	10	50				
	100	95.70502	87.92545	54.1329	64.9919	47.97407	56.56402	32.00972				
	100	89.57952	91.22486	56.76417	56.30713	59.23218	52.74223	34.27788				
	100	79.51654	97.83715	75.06361	74.68193	88.54962	45.6743	53.05344				
average	100	88.26703	92.32915	61.98689	65.32699	65.25195	51.66018	39.78035				
13-Jun poloxamer (%poloxamer in culturemedium) (23w:w)												
MDAMB231	no cell	1	2	3	4	5	6	7	8			
		0.027	1.026	0.887	1.075	0.82	0.801	0.783	0.505			
		-0.013	1.074	0.929	0.865	0.82	0.648	0.862	0.579			
		-0.014	0.84	1.046	0.659	0.707	0.649	0.785	0.624			
Survival ratio	0	0.0001	0.001	0.01	0.1	1	10					
	100	86.45224	104.7758	79.92203	78.07018	76.31579	49.22027					
	100	86.45224	80.54004	76.35009	60.3352	80.26071	53.91061					
	100	86.49907	78.45238	84.16667	77.2619	93.45238	74.28571					
average	100	86.46785	87.92275	80.14626	71.88909	83.34296	59.13887					
13-Jun poloxamer (%poloxamer in culturemedium) (23w:w)												
MCF-7/AL	no cell	1	2	3	4	5	6	7	8			
		0.023	1.154	0.773	1.236	0.851	0.71	0.846	0.45			
		-0.012	1.346	0.854	1.14	0.852	0.731	0.764	0.53			
		-0.011	1.329	0.852	0.811	0.725	0.873	0.826	0.694			
Survival ratio	0	0.0001	0.001	0.01	0.1	1	10					
	100	66.9844	107.1057	73.7435	61.52513	73.31023	38.9948					
	100	66.9844	84.69539	63.29866	54.30906	56.76077	39.37593					
	100	63.44725	61.02333	54.55229	65.68849	62.15199	52.21971					
average	100	65.80535	84.27481	63.86482	60.50756	64.07433	43.53015					

Sample Cytotoxicity Charts

MCF-7 survival



MDAMB435 survival

